



Molecular characterization of larval anisakid nematodes from marine fishes off the Moroccan and Mauritanian coasts

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ABSTRACT

A total of 242 larval forms of *Anisakis* collected from marine fishes at different sites off the Moroccan and Mauritanian coasts, recognised as belonging to Type I and Type II larvae, were identified by PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphisms) of the ITS (Internal Transcribed Spacers) region (ITS-1, 5.8 subunit rRNA gene and ITS-2), using a previously established molecular key. The Type I larvae were found with a frequency of 98.34% and were identified as belonging to the following species: *A. simplex* s.str., *A. pegreffii*, *A. simplex* s.str./*A. pegreffii* heterozygote genotypes, *A. typica*, *A. ziphidarum* and *Anisakis* sp. A. The Type II larvae were found to belong to *A. physeteris*, with the frequency of 1.65%. The results reported in the present study provide further epizootological and biological data on the *Anisakis* spp. in marine fishes off the Moroccan and Mauritanian coasts, improving the picture of the occurrence of these species in the central Atlantic coasts.

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1. Introduction

Anisakid nematodes have a global distribution among a wide variety of marine fish species that serve as intermediate or paratenic hosts [1]. Members of the genus *Anisakis* are of public health concern, as they may provoke serious gastrointestinal lesions in human, after the consumption of raw or undercooked parasitized fish [2]. In the last 20 years, the systematics and the taxonomic status of the species belonging to this genus have been largely clarified using genetic/molecular approaches. As a result, at least 8 taxa are currently recognized in the genus *Anisakis*. These include *A. simplex* s.str., *A. pegreffii*, *A. simplex* C, *A. typica*, *A. ziphidarum*, *A. physeteris*, *A. brevispiculata* and *A. paggiae* [3–9]. Two additional taxa, one identified by allozyme markers and mitochondrial *cox2* sequences and named provisionally *Anisakis* sp. [9], and one characterized by DNA sequencing of the nuclear ribosomal DNA spacers, provisionally named *Anisakis* sp. A [10], have been recently evidenced. *Anisakis* species are characterised by marked differences in their genetic structure as well as in ecological traits, such as geographic distributions and host preferences. Recently, the sequence analyses of nuclear

ribosomal DNA spacers have proved the existence of hybrid specimens between *A. pegreffii* and *A. simplex* s.str. [11]. The aim of the present study is to identify the *Anisakis* spp. in teleost fishes, using RFLP (Restriction Fragment Length Polymorphisms) genetic analysis and the molecular key devised by D'Amelio et al. [12], implemented by Pontes et al. [10], off the Moroccan and Mauritanian coasts and to establish their geographical distribution and host range in this area.

2. Materials and methods

A total of 242 larvae of anisakid nematodes were collected from different teleost fish species, captured at different sites off the Moroccan and Mauritanian coasts (North African coasts of Mediterranean Sea and East Atlantic Ocean) during April 2007 (Table 1, Fig. 1). The nematodes were repeatedly washed in 0.9% saline solution, fixed in 75% ethanol, and kept for later molecular identification. Total DNA was extracted using the DNA Purification Kit Wizard Genomic (Promega) according to the manufactures' instructions. DNA was eluted in 100 µl of elution buffer (10 mM Tris, 1 mM EDTA) and kept at –20 °C until use. PCR (Polymerase Chain Reaction) was conducted using a mixed solution of extracted DNA as a template (5 µl, 20–40 ng) and 50 µl reaction mixture containing 2.5 U AmpliTaq Gold (Applied Biosystems), 10 mM Tris–HCl (pH=8.3), 50 mM KCl (Applied Biosystems), 3 mM MgCl₂ (Promega), 1 mM of dNTPs (dCTP, dGTP, dATP, dTTP) (Promega) and 0.05 µM of each primer. For amplification of the nuclear rDNA

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Table 1Frequency (%) of *Anisakis* species larvae identified by PCR-RFLP from the sampling localities

Locality	Host species	Nh	Np	<i>A. pegreffii</i>	<i>A. simplex</i> s.str.	Heterozygote genotype	<i>A. typica</i>	<i>A. physeteris</i>	<i>A. ziphidarum</i>	<i>Anisakis</i> sp. A.
Mauritania										
Nouadhibou	<i>Merluccius merluccius</i>	17	19	47.4(9)	10.5(2)	5.3(1)	15.8(3)	5.3(1)	5.3(1)	10.5(2)
Morocco										
Laoune	<i>Euthynnus alleteratus</i>	1	12	41.6(5)	50.0(6)	8.4(1)	0.0	0.0	0.0	0.0
Casablanca	<i>Trachurus trachurus</i>	30	10	90.0(9)	0.0	10.0(1)	0.0	0.0	0.0	0.0
	<i>Trachurus mediterraneus</i>	18	10	90.0(9)	10.0(1)	0.0	0.0	0.0	0.0	0.0
	<i>Merluccius merluccius</i>	40	15	80.0(12)	6.7(1)	0.0	0.0	13.3(2)	0.0	0.0
	<i>Scomber scombrus</i>	22	12	41.7(5)	16.6(2)	16.6(2)	8.3(1)	0.0	8.3(1)	8.3(1)
	<i>Scomber japonicus</i>	10	18	61.1(11)	16.6(3)	5.5(1)	11.1(2)	0.0	0.0	5.5(1)
Rabat	<i>Merluccius merluccius</i>	13	9	55.5(5)	11.1(1)	0.0	11.1(1)	0.0	22.2(2)	0.0
Larache	<i>Merluccius merluccius</i>	20	20	55.0(11)	20.0(4)	5.0(1)	5.0(1)	5.0(1)	5.0(1)	5.0(1)
Tanger	<i>Trachurus trachurus</i>	26	28	78.6(22)	14.3(4)	7.1(2)	0.0	0.0	0.0	0.0
	<i>Trachurus mediterraneus</i>	14	14	78.6(11)	14.3(2)	7.1(1)	0.0	0.0	0.0	0.0
	<i>Scomber scombrus</i>	30	35	57.1(20)	31.4(11)	11.4(4)	0.0	0.0	0.0	0.0
	<i>Scomber japonicus</i>	18	13	76.9(10)	15.4(2)	7.7(1)	0.0	0.0	0.0	0.0
	<i>Merluccius merluccius</i>	35	17	70.6(12)	17.6(3)	11.8(2)	0.0	0.0	0.0	0.0
	<i>Lophius piscatorius</i>	7	6	100.0*(6)	0.0	0.0	0.0	0.0	0.0	0.0
Tetouan	<i>Merluccius merluccius</i>	3	4	100.0*(4)	0.0	0.0	0.0	0.0	0.0	0.0

Nh: Number of host examined.

Np: Number of specimens analysed by PCR-RFLP.

*These relative frequencies may be affected by the small sample size.

region containing ITS-1, 5.8S rRNA gene, and ITS-2, the previously described [13] primers NC5 (5'-GTA GGT GAA CCT GCG GAA GGA TCA TT-3') and NC2 (5'-TTA GTT TCT TTT CCT CCG CT-3') were used. The PCR was performed in a GeneAmp PCR System 2400 (Applied Biosystems) and the conditions were as follows: 10 min at 95 °C, then 30 cycles of 30 s at 95 °C, 30 s at 55 °C and 75 s at 72 °C followed by a final elongation of 7 min at 72 °C. A negative control (no DNA) was included in all PCR amplifications. Five microliters of the amplification products were visualised on 1% ethidium-bromide stained agarose gels to check the quality of amplification. The restriction enzymes, HinfI and HhaI, were used in RFLP analysis of rDNA for the identification of the members of *Anisakis* genus according to the genetic markers defined by D'Amelio et al. [12], implemented by Pontes et al. [10]. Restriction was performed using 10 µl of PCR products, 3 µl of distilled water, 0.5 µl of restriction enzyme, 1.5 µl of a specific buffer and 0.2 µl BSA up to a final volume of 15.2 µl. The digestion was performed for 90 min at 37 °C, and the digestion

products were evaluated on 2% ethidium-bromide-stained agarose gels. The PCR products of ribosomal DNA were purified using the commercial kit Nucleospin Extract (Macherey–Nagel) according to the manufactures' instructions. Sequencing was performed by MWG-BIOTECH AG (Ebersberg, Germany). Sequences were aligned with previously characterized sequences of *Anisakis* spp. using ClustalW [14] and adjusted manually. The electropherograms were analysed using Chromas 2.13.

3. Results

Two-hundred forty two *Anisakis* larvae, from 7 different fish species, were identified by PCR-RFLP, on the basis of the diagnostic restriction banding patterns. The amplification of the rDNA region (spanning the ITS-1, ITS-2 and the 5.8S subunit) produced a fragment of approximately 1000 bp. Two sibling species of the *Anisakis simplex* complex (*A. simplex* s.str. and *A. pegreffii*) and a heterozygote genotype

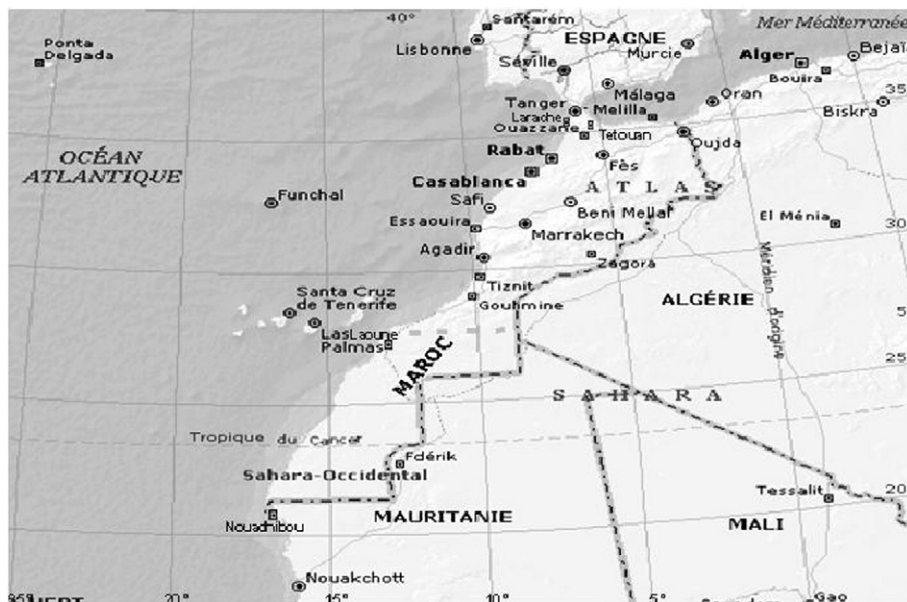


Fig. 1. Map showing the collecting sites of the *Anisakis* species larvae from Moroccan and Mauritanian coasts.

were identified using *Hinf*I (Fig. 2A), while digestion with *Hha*I produced the same pattern in these species (Fig. 2B). Four additional *Anisakis* species were also identified using the above enzymes [7,10], i.e., *A. physeteris*, *A. typica*, *Anisakis* sp. A. and *A. ziphidarum* (Fig. 2A, B). Digestion with *Hinf*I produced two strong bands (620 bp, 250 bp) and a weak band (80 bp) in *A. simplex* s.str., while *A. pegreffii* showed three bands of 370, 300 and 250 bp. Furthermore, the heterozygote genotype showed four fragments, corresponding to all the bands observed in *A. simplex* s.str. and in *A. pegreffii* (Fig. 2A). On the other hand, digestion with *Hha*I produced two fragments of 550 bp and 430 bp in *A. simplex* sensu lato and in heterozygotes (Fig. 2B). With *Hinf*I, *A. ziphidarum* exhibited 3 fragments (370–320–290 bp), *A. typica* exhibited 2 fragments (610–350 bp), *Anisakis* sp. A. exhibited 2 fragments (620–320 bp) and *A. physeteris* exhibited 3 bands (380–290–270 bp) (Fig. 2A). With *Hha*I, different restriction patterns were obtained for *A. ziphidarum*, *A. typica*, *A. physeteris* and *Anisakis* sp. A. (Bands of 550–430; bands of 320–240–180–160 bp; bands of 540–420 bp and 550–300–80 bp, respectively) (Fig. 2B). The alignment of the ITS-1–5.8S–ITS-2 sequences representing each of the *Anisakis* taxa, observed in this study, is shown in Fig. 3. One representative of each unique sequence was included for comparison. The consensus ITS-1–5.8S–ITS-2 sequences representing different *Anisakis* species were included in the alignment for comparative purposes. The ITS region of homozygote and heterozygote larval individuals, belonging to *A. simplex* complex, was further studied by sequence analysis. The sequence analyses revealed the presence of two polymorphic sites found at positions 244 and 260, corresponding to the sequence differences between *A. simplex* s.str. and *A. pegreffii*, at heterozygote status in some specimens, previously reported also by Abollo et al. [11] (Fig. 3). One out of the five sequences obtained for the individuals displaying the heterozygote genotype showed a nucleotide substitution (G instead of T) at position 321. Sequence comparison with available sequences confirmed the identification achieved by PCR-RFLP for all the species identified.

The Type I larvae (sensu Berland, [15]) were found with a frequency of 98.34% and were identified as belonging to the following species: *A. simplex* s.str., *A. pegreffii*, *A. simplex* s.str./*A. pegreffii* heterozygote genotypes, *A. typica*, *A. ziphidarum* and *Anisakis* sp. A. The Type II larvae (sensu Berland, [15]) were found to belong to *A. physeteris*, with a

frequency of 1.65%. From Tetouan, the only collecting site on the Mediterranean Sea, 100% of the larval forms examined were identified as *A. pegreffii*. From the East Atlantic Ocean, 65.96% of the larval forms examined were identified as *A. pegreffii*, 17.64% as *A. simplex* s.str., 7.14% as the heterozygote genotype, 1.68% as *A. physeteris*, 2.1% as *A. ziphidarum*, 3.36% as *A. typica* and 2.1% as *Anisakis* sp. A. (Table 1). The highest prevalence of heterozygote genotypes occurred in the North of the sampling area from the Tanger coasts, with a frequency of 58.82%. *A. simplex* s.str. often occurred parasitizing different fish species from several localities in the sampling area, but the highest frequencies were observed from Tanger coasts (52.38%) (Table 1).

4. Discussion

PCR-based restriction fragment length polymorphism (PCR-RFLP) analysis of the ribosomal DNA (rDNA) internal transcribed spacers (ITS-1 and ITS-2) provides a useful approach for the specific identification of both distantly and closely related ascaridoid species, showing high levels of interspecific sequence differences in the presence of low-level intraspecific variation [13]. Different studies have demonstrated that the ITS region (ITS-1, the 5.8S and the ITS-2) of nuclear rDNA provide useful genetic markers for the accurate identification of sibling species and morphospecies within *Anisakis* [10–12].

In the present study, different species of *Anisakis* were found parasitizing different fish species throughout the sampling area. The distribution patterns exhibited by the species of *Anisakis*, throughout their geographical range, are presented in Table 1. The results show that *A. pegreffii* is the most frequent species, in all fishing areas, parasitizing all the examined fish species. *A. pegreffii* has been previously reported as widely distributed from the Austral region, between the 35°S and the 55°S, as well as in the Mediterranean Sea [3]. The occurrence of this species, from the studied area, is consistent with its occurrence from the Southern Atlantic to Gibraltar Strait as suggested by Mattiucci et al. [3,17,18]. *A. simplex* s.str. is widespread between 35°N and the Arctic Polar Circle, mainly with a benthic or demersal life cycle [3,8,16].

The highest frequencies recorded for *A. simplex* s.str., in this study, were in samples from the waters off Tanger. In fact, according to Mattiucci et al. [3,8], *A. simplex* s.str. was the most prevalent species reported from the North-East Atlantic coasts, mainly from north of the

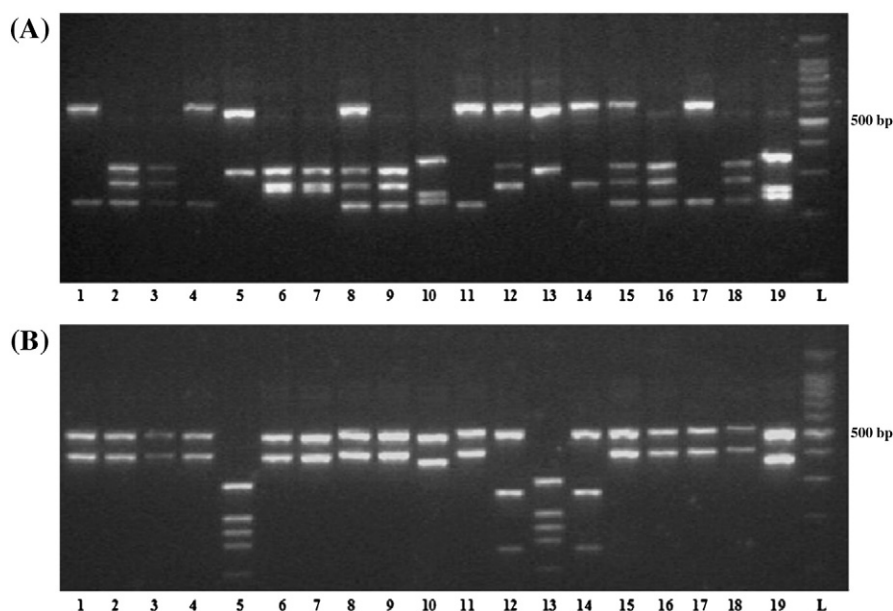


Fig. 2. RFLP profiles obtained by digestion of ITS region with the restriction enzymes *Hinf*I (A) and *Hha*I (B). Lanes: 1, 4, 11, 17 *A. simplex* s.str.; 2, 3, 9, 16, 18 *A. pegreffii*; 5, 13 *A. typica*; 6, 7 *A. ziphidarum*; 8, 15 Recombinant genotype; 10, 19 *A. physeteris*; 12, 14 *Anisakis* sp. A.; L 100 bp DNA ladder.

AY826723	ACCTTCCATT	TGCATGTTGT	TGTGAGCCAC	ATGGAAACTC	GTACACA--C	GTGGTGCC--	[60]
AY826720							[60]
AY826721	..G.....		..G.....		A.....-T		[60]
AY826724	..GC.....		..G.....	..C.....	GTTT	TGA	[60]
AY826722							[60]
AY260555	..G.....		..G.....		CAT		[60]
AY826725	..G.....		..G.....		-T		[60]
HG							[60]
A. simplex s.str							[60]
A. pegreffii							[60]
A. physeteris	..G.....		..G.....		A.....-T		[60]
A. typica	..GC.....		..G.....	..C.....	GTTT	TGA	[60]
Anisakis sp. A	..G.....		..G.....		CAT		[60]
A. ziphidarum	..G.....		..G.....		-T		[60]
AY826723	-AGCCGCTCTG	CTGTGCTTTT	TTTAGGCAGA	CAATGGCTTA	CGAGTGGC-C	GTGT-GCTTG	[120]
AY826720							[120]
AY826721		A	..-GT.....		T.....	C..	[120]
AY826724	T.....	G..C	G..G.....		CT	CC..	[120]
AY826722							[120]
AY260555			..-GT.....		A..		[120]
AY826725			..-GT.....		-T		[120]
HG							[120]
A. simplex s.str							[120]
A. pegreffii							[120]
A. physeteris		A	..-GT.....		T.....	C..	[120]
A. typica	T.....	G..C	G..G.....		-T	C..	[120]
Anisakis sp. A			..-GT.....		A..		[120]
A. ziphidarum			..-GT.....		-T		[120]
AY826723	TTGAACAACG	GTGACCAATT	TGGC-GTCTA	CGCCGTATCT	AGCTTCTGCC	TGGACCGTCA	[180]
AY826720							[180]
AY826721							[180]
AY826724			..T.....		C..	G	[180]
AY826722							[180]
AY260555						G	[180]
AY826725					C..	G	[180]
HG							[180]
A. simplex s.str							[180]
A. pegreffii							[180]
A. physeteris							[180]
A. typica					C..	G	[180]
Anisakis sp. A						G	[180]
A. ziphidarum					C..	G	[180]
AY826723	GTTCGATGA	AAGATGCGGA	GAAAGTTCCT	TTGTTTGGC	TGCTAATCAT	CATTGATGAG	[240]
AY826720							[240]
AY826721	..A.....			C..	A..	CG..G.	[240]
AY826724	..A.....		..G.....	C..T			[240]
AY826722							[240]
AY260555	..A.....	A..	..G.....	A	-T.....	A..	[240]
AY826725			..G.....		-T.....	C..	[240]
HG							[240]
A. simplex s.str							[240]
A. pegreffii							[240]
A. physeteris	..A.....			C..	A..	CG..G.	[240]
A. typica	..A.....		..G.....	C..T			[240]
Anisakis sp. A	..A.....	A..	..G.....	A	-T.....	A..	[240]
A. ziphidarum			..G.....		-T.....	C..	[240]
AY826723	CAGTAGCTTA	AGGCAGAGTT	GAGCAGACTT	AATGAGCCAC	GCT--AGGTG	GCCGCCAAAA	[300]
AY826720	..C.....	C					[300]
AY826721	-----CC..	..CA..G..C			-T.....		[300]
AY826724	-----	..G.....			CT.....	G..	[300]
AY826722	..C.....						[300]
AY260555	-----G	C					[300]
AY826725	-----C.G	..T.....C					[300]
HG	..Y.....	Y					[300]
A. simplex s.str							[300]
A. pegreffii	..C.....	C					[300]
A. physeteris	-----CC..	..CA..G..C			-T.....		[300]
A. typica	-----	..G.....			CT.....	G..	[300]
Anisakis sp. A	-----G	C					[300]
A. ziphidarum	-----C.G	..T.....C					[300]
AY826723	CCCAAAACAC	AACCGGTCTA	TTTGACATTG	TTATTTTCATT	GTATGTGTTG	AA--AATGTA	[360]
AY826720							[360]
AY826721		A.....		CAG.ATGC	..TGACA..A	CGTT.....	[360]
AY826724		-----C	A...TTG.CA	..T-GA.....	..TGA..A..	..T--T.....	[360]
AY826722							[360]
AY260555				A.T.....			[360]
AY826725				-T.....			[360]
HG							[360]
A. simplex s.str							[360]
A. pegreffii							[360]
A. physeteris		A.....		CAG.ATGC	..TGACA..A	CGTT.....	[360]
A. typica		-----C	A...TTG.CA	..T-GA.....	..TGA..A..	..T--T.....	[360]
Anisakis sp. A				A.T.....			[360]
A. ziphidarum				-T.....			[360]
AY826723	CAAATCTTGG	CGGTGGATCA	CTCGGTTTCGT	GGATCGATGA	AGAACGCAGC	CAGCTGCGAT	[420]
AY826720							[420]
AY826721			T.....				[420]
AY826724							[420]
AY826722							[420]

Fig. 3. Alignment of ITS-1–5.8S–ITS-2 sequences of *Anisakis* taxa. One representative of each unique sequence was included for comparison. Dots indicate identity with the first sequence and dashes are inferred insertion–deletion events; Standard IUB ambiguity codes are used, i.e., Y = C/T, R = A/G. The consensus ITS-1–5.8S–ITS-2 sequences for the *A. pegreffii* (AY826720), *A. simplex* s.str. (AY826723), *A. simplex* C (AY826722), *A. typica* (AY826724), *A. ziphidarum* (AY826725), *A. physeteris* (AY826721) and *Anisakis* sp. A. (AY260555) were included in the alignment for comparative purposes. The heterozygote genotype is indicated as HG.

Fig. 3 (continued).

AY826723	AGCATACG--	-----TG	ATAAGTTGGC	TGGTTGATGA	AACGGCAACG	GAATGACGGA	[840]
AY826720							[840]
AY826721			..GT.....		CT.TTGG.A	ACGGAGT.A-	[840]
AY826724	TC	TATGATAC.A	G..G.....			TGC..C	[840]
AY826722							[840]
AY260555	G-	-----A				TAT.	[840]
AY826725	A-	-----				T--	[840]
HG							[840]
<i>A. simplex</i> s.str							[840]
<i>A. pegreffii</i>							[840]
<i>A. physeteris</i>			..GT.....		CT.TTGG.A	ACGGAGT.A-	[840]
<i>A. typica</i>							[840]
<i>Anisakis</i> sp. A		-----A				TAT.	[840]
<i>A. ziphidarum</i>		-----A				T--	[840]
AY826723	CGTCTATG?G	ATCAAAAATG	ATACTATTG	ACCTC----	-AGCTCAGTC	GTGATTACCC	[900]
AY826720	T						[900]
AY826721	---GG..T	...GG..C	T.TG.....				[900]
AY826724	ATG.ATGTGA	TCG.G..GC	...A.G..C	TAT.TGACCT	C.....		[900]
AY826722	...T.....						[900]
AY260555	..G...T	A	T.TA.....				[900]
AY826725	--G...T		T.TA.....				[900]
HG	T						[900]
<i>A. simplex</i> s.str	T						[900]
<i>A. pegreffii</i>							[900]
<i>A. physeteris</i>	---GG..T	...GG..C	T.TG.....				[900]
<i>A. typica</i>							[900]
<i>Anisakis</i> sp. A	..G...T	A	T.....				[900]
<i>A. ziphidarum</i>	--G...T		T.TA.....				[900]
AY826723	GCTGAATTGA	AGC	[913]				
AY826720			[913]				
AY826721			[913]				
AY826724			[913]				
AY826722			[913]				
AY260555			[913]				
AY826725			[913]				
HG			[913]				
<i>A. simplex</i> s.str			[913]				
<i>A. pegreffii</i>			[913]				
<i>A. physeteris</i>			[913]				
<i>A. typica</i>			[913]				
<i>Anisakis</i> sp. A			[913]				
<i>A. ziphidarum</i>			[913]				

Fig. 3 (continued).

Straits of Gibraltar. Its occurrence off the Spanish Atlantic coast has been previously documented, as its sympatric occurrence in this area with *A. pegreffii* [3,7,11,17], and its presence at adults stage in *Delphinus delphis* L. [11]. The findings here obtained confirm the occurrence of *A. simplex* s.str. in the Atlantic coasts of North Africa, as reported by Mattiucci et al. [8,17,18]. Our data showed that 83% of the fish species infected by *A. simplex* s.str. are pelagic. This species was also previously documented from the Madeiran and Galician waters in pelagic fishes, such as *Belone belone*, *Micromesistius poutassou*, *Scomber scombrus*, *Scomber japonicus*, *Trachurus trachurus* and *Trachurus picturatus* [10,11,16].

The sequence analyses of the ribosomal DNA of *A. simplex* s.str. and *A. pegreffii* revealed the presence of two polymorphic sites found at positions 244 and 260, corresponding to the sequence differences between them, at heterozygote status in some specimens. The significance of the occurrence of a variant specimen, showing a nucleotide substitution (G instead of T) at position 321, needs to be further investigated. The record of *A. simplex* s.str./*A. pegreffii* heterozygotes, previously recorded from the Spanish and Portuguese Atlantic coasts and the Alboran Sea, extends their geographic distribution to the Moroccan and Mauritanian coasts.

According to Mattiucci et al. [7], the range of *A. typica* extends from 30°S to 35°N in warmer temperate and tropical waters. This species was previously documented from the Mediterranean Sea, the Moroccan and Portuguese Atlantic coasts [7,8,17,19]. Most of the cetaceans reported in literature as definitive hosts of *A. typica* belong to the families Delphinidae, Phocoenidae and Pontoporidae [7]. Its typical definitive hosts are common in the epipelagic region [10], thus explaining the occurrence of this species in pelagic fishes as suitable paratenic host, such as *Scomber scombrus* and *S. japonicus*, although it has been found also in a demersal species (*M. merluccius*). The present study represents, therefore, an additional record of *A. typica* from the Moroccan and the Mauritanian coasts.

A. ziphidarum, which occurred, in this study, in both hake (80%) and mackerel (20%), can indeed be found in both the pelagic and the deepwater regions [10]; its definitive hosts, *Mesoplodon layardii* and *Ziphius cavirostris* [6], were previously reported from the Madeiran waters, the Atlantic coast of Morocco and the Mediterranean Sea [20]. As to *A. physeteris*, its main definitive host is the sperm whale, *Physeter catodon*. At larval stages, *A. physeteris* is typical of epipelagic paratenic hosts of the Mediterranean and central Atlantic Ocean [4,17,21,22]. *A. ziphidarum* and *A. physeteris* were previously identified in *M. merluccius* from the Moroccan [17] and the Mauritanian coasts [8]. *Anisakis* sp. A. was reported for the first time in *Aphanopus carbo* and *S. japonicus* from the Madeiran waters [10]. This is the first record of *Anisakis* sp. A. larvae from the Moroccan and Mauritanian coasts as well as *M. merluccius* and *S. scombrus* represent new paratenic hosts for larvae of this species. The final host of this taxon could possibly be a member of the family Ziphiidae, as suggested by Pontes et al. [10] on the basis of its close relationship with *A. ziphidarum*, inferred by sequence comparison of nuclear rDNA spacers.

The occurrence of different species of *Anisakis* from the East Atlantic Ocean may be due to the presence in this area of various species of dolphins, sperm whales, beaked whales and Blainville's beaked whale, which represent the definitive hosts for *A. simplex* s.str. and *A. pegreffii*, *A. physeteris*, *A. ziphidarum* and *Anisakis* sp. A., respectively. In this study, we provide further data on the frequency of *Anisakis* spp. in marine fishes off the Moroccan and Mauritanian coasts, improving the picture of the occurrence of these species in the central Atlantic coasts.

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